

Figure S1. TH positive cells, additional clusters.

Number of TH-positive cells in supra-chiasmatic nucleus, olfactory bulb and subpallium, locus coeruleus and medulla oblongata, for SF and CF, both mutant homozygotes (m) or non-mutant (+). Non-mutant SF (+) tested are either wildtype (first bar), or mutants siblings (second bar).

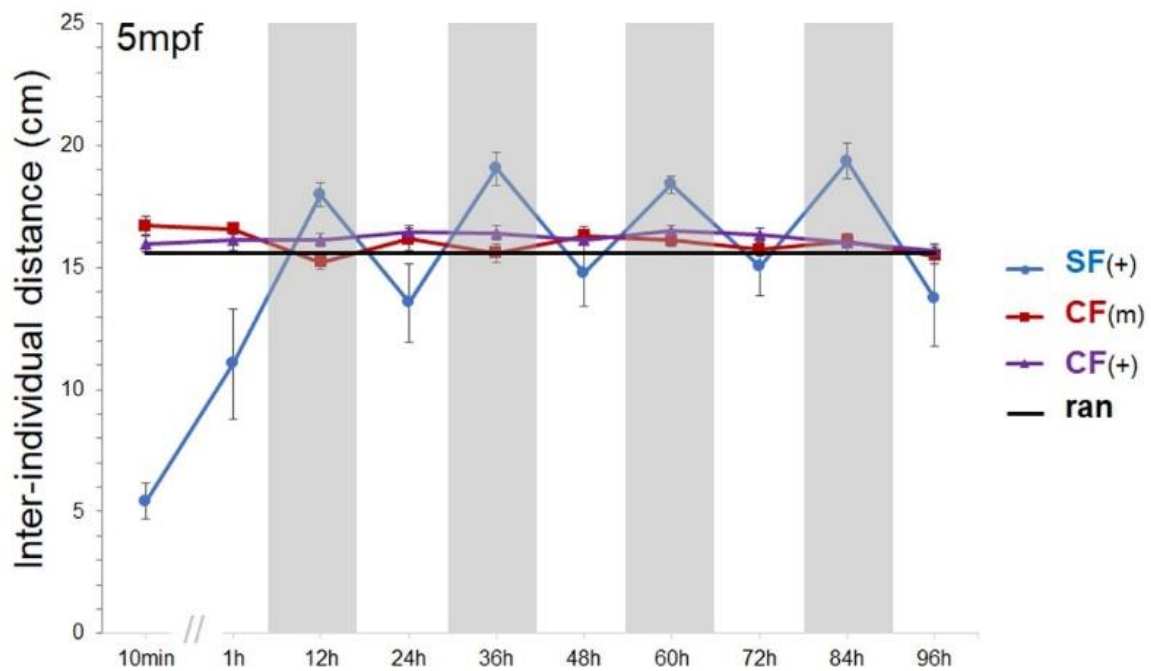
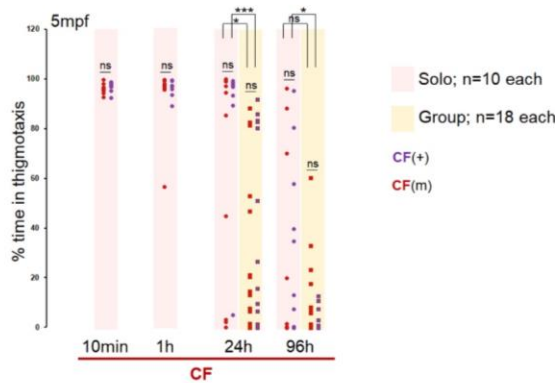


Figure S2. Shoaling in SF and CF, measured over a one week period.

Mean of inter-individual distance (IID) measured during the day or during the night, over 5 days in groups of 6 fish: n=10 groups for P106L mutant CF (red line), n=12 groups for non-mutant CF (purple line), and n=5 groups for non-mutant SF (blue line). The black line is the mean value of inter-individual distance obtained with 100.000 simulations of random distribution of fish (ran).

A. Stress behavior in 5mpf cavefish: thigmotaxis



B. Stress behaviors in 5mpf surface fish

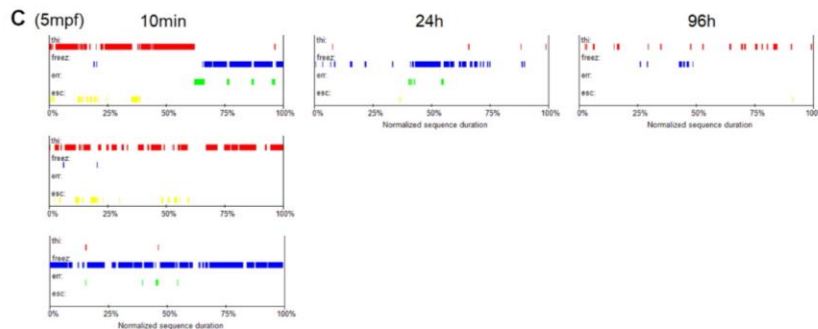
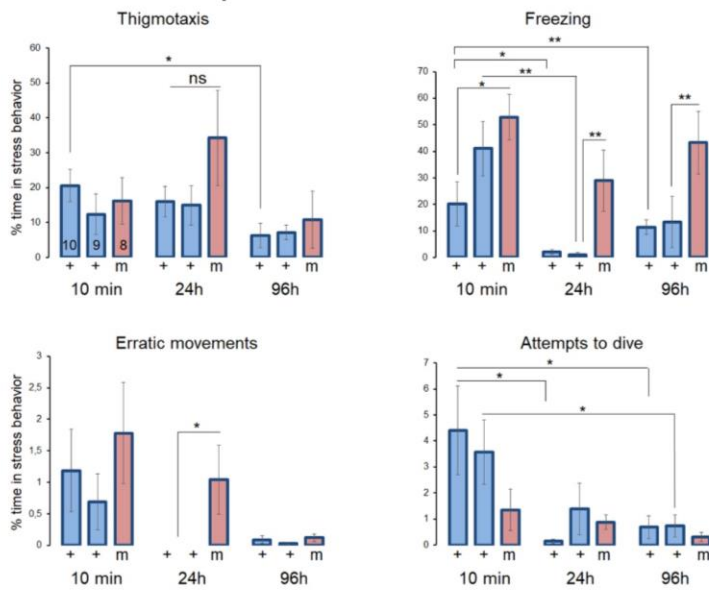


Figure S3. Stress behaviors in 5mpf CF and SF.

(A) Thigmotaxis in CF. Measurements of the percentage of time doing thigmotaxis 10min, 1h, 24h or 96h after the fish was/were put alone (pink shade) or in group (yellow shade) in a novel environment. The measurements were performed on P106L mutant CF (red) and non-mutant CF (purple), aged 5 months. Data are presented as scattered plots to represent the inter-individual variability of this phenotype at the two later time-points.

At 10min and 1h after transfer in a new tank, CF spent ~100% of their time doing thigmotaxis, regardless of their *mao* genotype. It was impossible to score groups of fish at these times (i.e., no

yellow bars for 10min and 1h) because frenetic swimming led to loss of the track of individual fish (manual scoring).

At 24h and 96h after transfer in a new tank, data could be obtained for the 4 conditions: mutant and non-mutant, solo or groups. As inter-individual variability was high, there was no detectable significant effect of the *mao* mutation on thigmotaxis behavior, either alone or in group. However and interestingly, there was a significant reduction of thigmotaxis, for both the mutant and the non-mutant CF, when the fish were in groups as compared to solo. This may suggest that the group has an anxiolytic effect on CF, although the CF morphotype of *A. mexicanus* is often described as a “non-social” animal because it does not show collective behavior such as schooling or shoaling. Of note, we cannot rule out the possibility that the “anxiolytic effect” observed here was not due to the size of the tank. Indeed, groups were tested in larger tanks (40x23cm; 5l) than single fish (19x10cm; 600ml), hence the possibility of a confinement stress in the later.

(B) Stress behavior repertoire in SF. Measurements of the percentage of time doing thigmotaxis, freezing, erratic movements and attempts to dive. The measurements were performed on P106L mutant SF (m) and non-mutant SF (+), and 10min, 24h or 96h after the fish was put alone in a novel environment. Non-mutant SF tested were either wt (first bar of each group), or siblings of mutants SF (second bar).

(C) Representative ethograms for SF, showing the alternation of periods where the fish display thigmotaxis (‘thi’ in red), freezing (‘freez’ in blue), erratic movements (‘err’ in green) and attempts to dive (‘esc’ in yellow). The 3 ethograms of the top line belong to the same fish, recorded 10min, 24h and 96h after being put in a novel environment. The 3 ethograms of the first column belong to 3 different fish recorded 10min after being put in a novel environment. Note the individual preferences for a given behavior, which can also vary along time for a single individual. Hence, the large error bars on graphs in A, and the necessity to combine all stress behaviors for analysis (as shown in Fig. 7B).

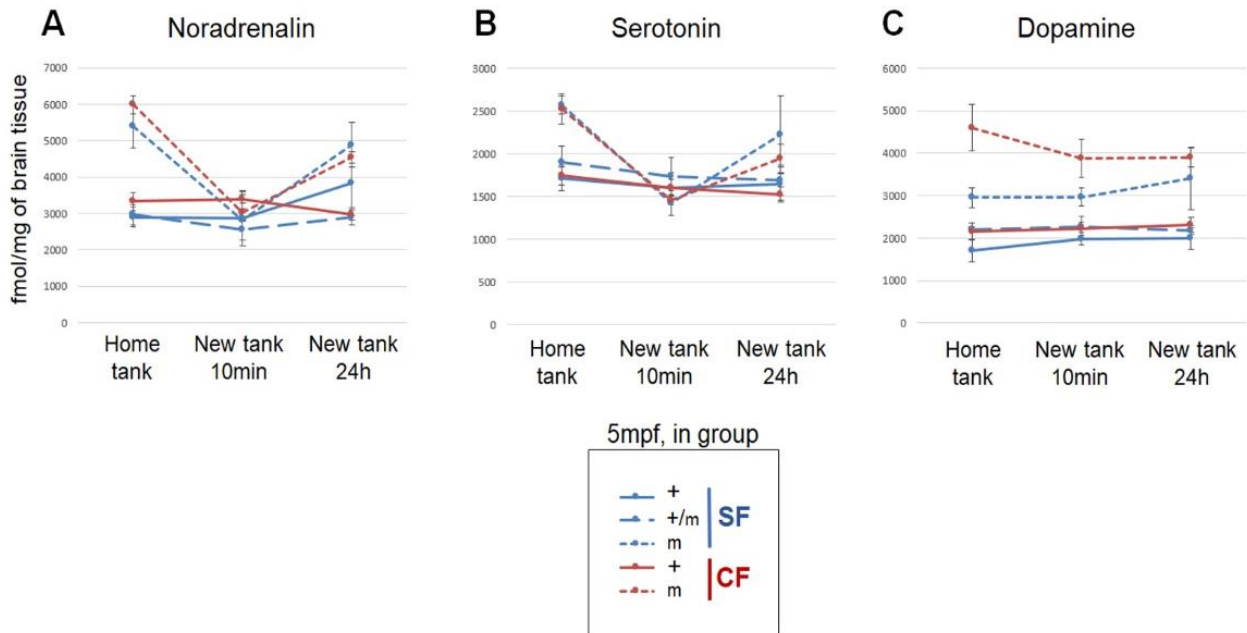


Figure S4. Levels of serotonin, noradrenaline and dopamine in the brain before, during and after a stressor (novel environment), in 5mpf fish in groups.

Noradrenaline (A), serotonin (B) and dopamine (C) measurements in the brains SF and CF, both P106L mutant homozygotes (m, dotted lines) and non-mutant (+, continuous lines), and heterozygotes SF (+/m, long dotted lines). Measurements were performed on fish held in groups of 6 in their home tank (HT) at the fish facility, in a novel tank for 10 min (NT10), and in a novel tank for 24h (NT24). Only mutants show strong variations of brain NA and 5-HT after stress.

Statistics for 5-HT: HT mCF versus HT +CF $p=0.0011$; HT mSF versus HT +SF $p=0.0159$; HT mCF versus NT10 mCF $p=0.0022$; HT mSF versus NT10 mSF $p=0.0079$; NT24 mCF versus NT24 +CF $p=0.0312$.

Statistics for NA: HT mSF versus HT +SF $p=0.0159$; HT mSF versus HT m/+SF $p=0.0079$; HT mCF versus HT +CF $p=0.0002$; HT mCF versus NT10 mCF $p=0.0022$; HT mCF versus NT24 mCF $p=0.0022$; NT10 mCF versus NT24 mCF $p=0.0022$; NT24 mSF versus NT24 m/+SF $p=0.0159$; NT24 mCF versus NT24 +CF $p=0.0002$.

Statistics for DA: HT mSF versus HT +SF $p=0.0317$; HT mSF versus HT m/+SF $p=0.0079$; HT mCF versus HT +CF $p=0.0031$; NT10 mSF versus NT10 +SF $p=0.0159$; NT10 mCF versus NT10 +CF $p=0.0002$; NT24 mCF versus NT24 +CF $p=0.0010$.

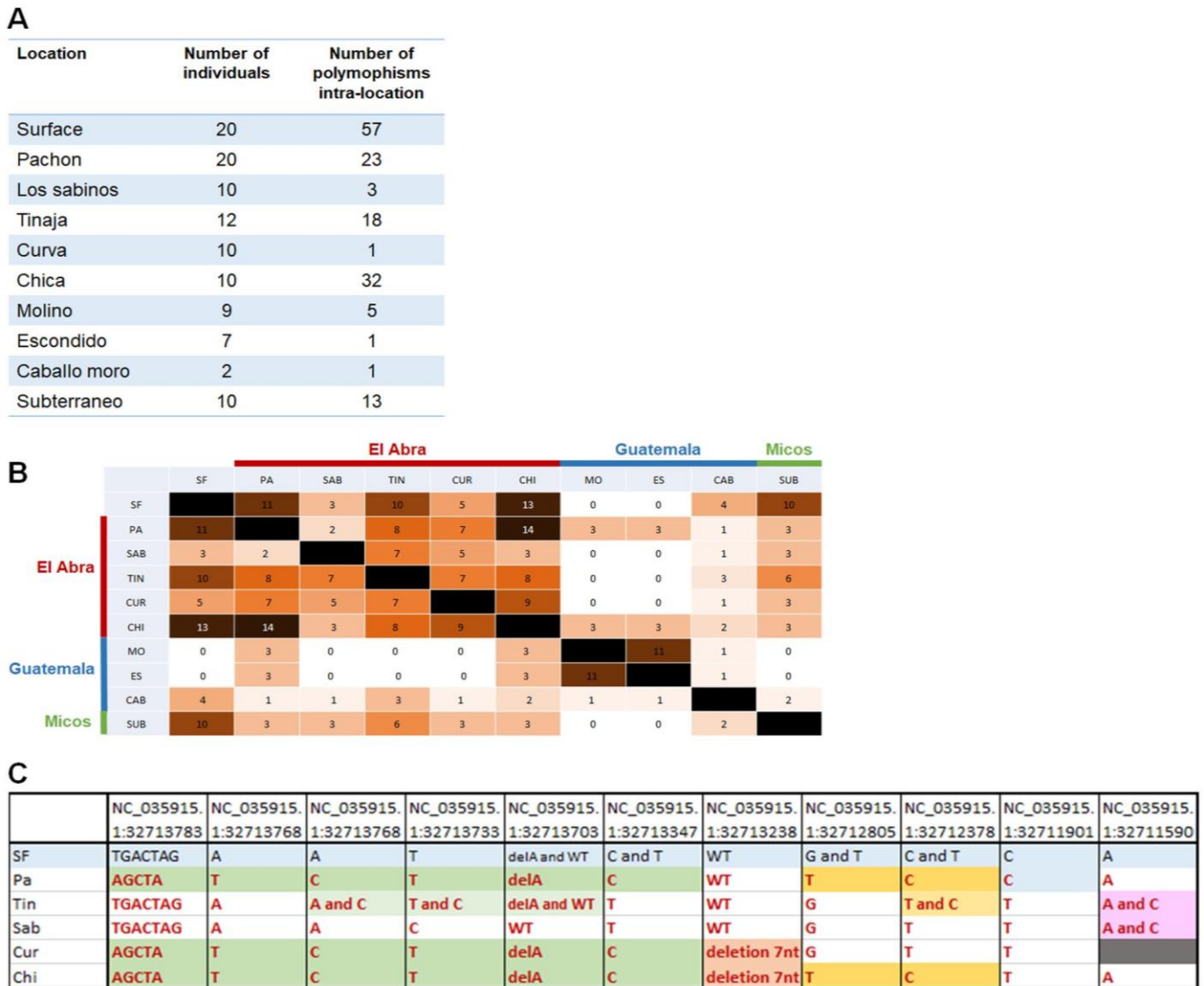


Figure S5. Polymorphism along a ~4 kb fragment of the *mao* gene, and *mao* haplotypes.

(A) Number of wild-sampled individuals tested and number of polymorphisms in the ~4kb fragment around the P106L position in the *mao* gene, detected at each sampling site. For instance, we sequenced 20 fish from the river population, and we found 57 polymorphic positions distributed along the sequenced fragment. (B) Number of shared polymorphisms between surface populations and cave populations from El Abra, Guatemala and Micos groups. Darker colors indicate a high number of shared polymorphisms. Note that they are mostly shared within groups of caves. (C) Haplotypes reconstitution

for the P106L mutant allele. Each column corresponds to a polymorphic position. The first line depicts the haplotypes encountered in SF (all Proline at position 106 – homozygote wildtype), and the next lines are haplotypes associated with the Leucine106 (P106L) mutant allele in cave populations of the El Abra group. For instance, all P106L mutant individuals sequenced from Pachón cave had the sequence AGCTA (first column in the table) at the position 32713783, whereas all P106L mutants from the Tinaja cave had the sequence TGACTAG instead at that same position. The P106L mutation is in position NC_035915.1:32711472.

Table S1. Summary statistics for figure 7.

Three-way ANOVA		Ordinary			
Alpha		0,05			
Source of Variation	% of total variation	P value	P value summary	Significant?	
Habituation	46,51	<0.0001	****	Yes	
Morphotype	6,266	<0.0001	****	Yes	
Genotype	11,51	<0.0001	****	Yes	
Habituation x Morphotype	1,704	0,0223	*	Yes	
Habituation x Genotype	21,09	<0.0001	****	Yes	
Morphotype x Genotype	1,473	0,0104	*	Yes	
Habituation x Morphotype x Genotype	1,329	0,0497	*	Yes	
ANOVA table					
Habituation	SS (Type III)	DF	MS	F (DFn, DFd)	P value
Habituation	263092	2	131546	F (2, 65) = 110.1	P<0.0001
Morphotype	35448	1	35448	F (1, 65) = 29.66	P<0.0001
Genotype	65090	1	65090	F (1, 65) = 54.46	P<0.0001
Habituation x Morphotype	9639	2	4820	F (2, 65) = 4.033	P=0.0223
Habituation x Genotype	119278	2	59639	F (2, 65) = 49.90	P<0.0001
Morphotype x Genotype	8333	1	8333	F (1, 65) = 6.972	P=0.0104
Habituation x Morphotype x Genotype	7516	2	3758	F (2, 65) = 3.145	P=0.0497
Residual	77682	65	1195		
Posthoc					
Holm-Sidak's multiple comparisons test	Mean Diff.	Significant?	Summary	Adjusted P Value	
HT:SF+ vs. HT:SFm	18,34	No	ns	0,9988	
HT:SF+ vs. HT:CF+	13,79	No	ns	0,9988	
HT:SF+ vs. HT:CFm	30,56	No	ns	0,9544	
HT:SF+ vs. NT 10min:SF+	-46,66	No	ns	0,6957	
HT:SF+ vs. NT 10min:SFm	-271,1	Yes	****	<0.0001	
HT:SF+ vs. NT 10min:CF+	-41,82	No	ns	0,6601	
HT:SF+ vs. NT 10min:CFm	-167,7	Yes	****	<0.0001	
HT:SF+ vs. NT 24h:SF+	-48,92	No	ns	0,6957	
HT:SF+ vs. NT 24h:SFm	-95,7	Yes	**	0,0087	
HT:SF+ vs. NT 24h:CF+	3,265	No	ns	0,9996	
HT:SF+ vs. NT 24h:CFm	-7,198	No	ns	0,9996	
HT:SFm vs. HT:CF+	-4,555	No	ns	0,9996	
HT:SFm vs. HT:CFm	12,22	No	ns	0,9988	
HT:SFm vs. NT 10min:SF+	-65	No	ns	0,1205	
HT:SFm vs. NT 10min:SFm	-289,5	Yes	****	<0.0001	
HT:SFm vs. NT 10min:CF+	-60,16	No	ns	0,0661	
HT:SFm vs. NT 10min:CFm	-186,1	Yes	****	<0.0001	
HT:SFm vs. NT 24h:SF+	-67,26	No	ns	0,1417	
HT:SFm vs. NT 24h:SFm	-114	Yes	***	0,0003	
HT:SFm vs. NT 24h:CF+	-15,08	No	ns	0,9988	
HT:SFm vs. NT 24h:CFm	-25,54	No	ns	0,9789	
HT:CF+ vs. HT:CFm	16,77	No	ns	0,9957	
HT:CF+ vs. NT 10min:SF+	-60,45	No	ns	0,0653	
HT:CF+ vs. NT 10min:SFm	-284,9	Yes	****	<0.0001	
HT:CF+ vs. NT 10min:CF+	-55,61	Yes	*	0,0136	
HT:CF+ vs. NT 10min:CFm	-181,5	Yes	****	<0.0001	
HT:CF+ vs. NT 24h:SF+	-62,7	No	ns	0,0885	
HT:CF+ vs. NT 24h:SFm	-109,5	Yes	****	<0.0001	
HT:CF+ vs. NT 24h:CF+	-10,53	No	ns	0,9988	
HT:CF+ vs. NT 24h:CFm	-20,99	No	ns	0,9789	
HT:CFm vs. NT 10min:SF+	-77,22	Yes	*	0,0174	
HT:CFm vs. NT 10min:SFm	-301,7	Yes	****	<0.0001	
HT:CFm vs. NT 10min:CF+	-72,38	Yes	**	0,0045	
HT:CFm vs. NT 10min:CFm	-198,3	Yes	****	<0.0001	
HT:CFm vs. NT 24h:SF+	-79,48	Yes	*	0,0254	
HT:CFm vs. NT 24h:SFm	-126,3	Yes	****	<0.0001	
HT:CFm vs. NT 24h:CF+	-27,3	No	ns	0,9082	
HT:CFm vs. NT 24h:CFm	-37,76	No	ns	0,7277	
NT 10min:SF+ vs. NT 10min:SFm	-224,5	Yes	****	<0.0001	
NT 10min:SF+ vs. NT 10min:CF+	4,839	No	ns	0,9996	
NT 10min:SF+ vs. NT 10min:CFm	-121,1	Yes	****	<0.0001	
NT 10min:SF+ vs. NT 24h:SF+	-2,256	No	ns	0,9996	
NT 10min:SF+ vs. NT 24h:SFm	-49,04	No	ns	0,6376	
NT 10min:SF+ vs. NT 24h:CF+	49,92	No	ns	0,2551	
NT 10min:SF+ vs. NT 24h:CFm	39,46	No	ns	0,7277	
NT 10min:SFm vs. NT 10min:CF+	229,3	Yes	****	<0.0001	
NT 10min:SFm vs. NT 10min:CFm	103,4	Yes	***	0,0003	
NT 10min:SFm vs. NT 24h:SF+	222,2	Yes	****	<0.0001	
NT 10min:SFm vs. NT 24h:SFm	175,4	Yes	****	<0.0001	
NT 10min:SFm vs. NT 24h:CF+	274,4	Yes	****	<0.0001	
NT 10min:SFm vs. NT 24h:CFm	263,9	Yes	****	<0.0001	
NT 10min:CF+ vs. NT 10min:CFm	-125,9	Yes	****	<0.0001	
NT 10min:CF+ vs. NT 24h:SF+	-7,095	No	ns	0,9996	
NT 10min:CF+ vs. NT 24h:SFm	-53,88	No	ns	0,2437	
NT 10min:CF+ vs. NT 24h:CF+	45,08	No	ns	0,1203	
NT 10min:CF+ vs. NT 24h:CFm	34,62	No	ns	0,6962	
NT 10min:CFm vs. NT 24h:SF+	118,8	Yes	****	<0.0001	
NT 10min:CFm vs. NT 24h:SFm	72,04	No	ns	0,0661	
NT 10min:CFm vs. NT 24h:CF+	171	Yes	****	<0.0001	
NT 10min:CFm vs. NT 24h:CFm	160,5	Yes	****	<0.0001	
NT 24h:SF+ vs. NT 24h:SFm	-46,79	No	ns	0,7275	
NT 24h:SF+ vs. NT 24h:CF+	52,18	No	ns	0,2994	
NT 24h:SF+ vs. NT 24h:CFm	41,72	No	ns	0,7277	
NT 24h:SFm vs. NT 24h:CF+	98,97	Yes	***	0,0004	
NT 24h:SFm vs. NT 24h:CFm	88,51	Yes	**	0,0075	
NT 24h:CF+ vs. NT 24h:CFm	-10,46	No	ns	0,9988	

Mann-Whitney pairwise comparisons

	SF + NT10	SF + NT24	SF +/- anim	SF +/- NT10	SF +/- NT24	SF m HT	SF m NT10	SF m NT24	CF m HT	CF m NT10	CF m NT24	CF + HT	CF + NT10	CF + NT24													
SF + HT	0.111																										
SF + NT10		0.057																									
SF + NT24			0.412																								
SF +/- HT				0.007																							
SF +/- NT10					0.555																						
SF +/- NT24						0.222																					
SF m HT							0.0555																				
SF m NT10								0.190																			
SF m NT24									0.015																		
CF m HT										0.008																	
CF m NT10											0.004																
CF m NT24												0.019															
CF + HT													0.137														
CF + NT10														0.661													
CF + NT24															0.007												
SF m HT																0.008											
SF m NT10																	0.0006										
SF m NT24																		0.0004									
CF m HT																			0.0019								
CF m NT10																				0.002							
CF m NT24																					0.002						
CF + HT																						0.0001					
CF + NT10																								0.219			
CF + NT24																									0.173		
CF + HT																										0.000034	
CF + NT10																											0.173
CF + NT24																											0.002

	SF + NT10	SF + NT24	SF +/- anim	SF +/- NT10	SF +/- NT24	SF m HT	SF m NT10	SF m NT24	CF m HT	CF m NT10	CF m NT24	CF + HT	CF + NT10	CF + NT24	
SF + HT	NS														
SF + NT10		NS													
SF + NT24			NS												
SF +/- HT				**											
SF +/- NT10					NS										
SF +/- NT24						NS									
SF m HT							**								
SF m NT10								*							
SF m NT24									NS						
CF m HT										NS					
CF m NT10											**				
CF m NT24												**			
CF + HT													*		
CF + NT10														***	
CF + NT24															***
CF + HT															NS
CF + NT10															NS
CF + NT24															**

one-way ANOVA for each group, along time

SF + Statistique observée Qobs : 4.8660986357807

p-value : 0.033428951624457 *

Test d'homogénéité de variance de Bartlett

Méthode : Bartlett test of homogeneity of variances
Statistique observée Qobs : 2.5648540679301
p-value : 0.27736331270786
Paramètre du test : 2

SF +/- Statistique observée Qobs : 15.190245363751

p-value : 0.00051533731390284 ***

Test d'homogénéité de variance de Bartlett

Méthode : Bartlett test of homogeneity of variances
Statistique observée Qobs : 5.3132918848822
p-value : 0.07018322599511
Paramètre du test : 2

SF m Statistique observée Qobs : 30.287214416875

p-value : 3.3611768813376E-5 ***

Test d'homogénéité de variance de Bartlett

Méthode : Bartlett test of homogeneity of variances
Statistique observée Qobs : 16.998091696251
p-value : 0.00020366260138521
Paramètre du test : 2

CF + Statistique observée Qobs : 16.140270446008

p-value : 1.9435396964723E-5 ***

Test d'homogénéité de variance de Bartlett

Méthode : Bartlett test of homogeneity of variances
Statistique observée Qobs : 7.3937770507278
p-value : 0.02480057289771
Paramètre du test : 2

CF m Statistique observée Qobs : 64.137389491156

p-value : 4.4607854863108E-8 ***

Test d'homogénéité de variance de Bartlett

Méthode : Bartlett test of homogeneity of variances
Statistique observée Qobs : 19.814791926837
p-value : 4.9804960850732E-5
Paramètre du test : 2



Movie 1: thigmotaxis in CF, 10min after being placed in a novel tank.



Movie 2: thigmotaxis in CF, 1hour after being placed in a novel tank.



Movie 3: thigmotaxis in CF, 96hours after being placed in a novel tank.